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TI Test paper for determination of **hydrogen peroxide** content
IN Ono, Masayuki; Ohno, Yasuaki; Morita, Kiyoshi
PA Eiken Chemical Co., Ltd., Japan
SO Japan., 4 pp.
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DT Patent
LA Japanese
AB A testing paper for the detn. of H_2O_2 consists of a filter paper treated, for .apprx.30 sec, with a mixt. of H_2O-decomposing enzyme (peroxidase or catalase), o-tolidine-EtOH, Acid **Fuchsine**, and citrate buffer. The paper is dried in the dark, and treated with the sample for 20 sec. The color change of the paper is compared with stds. to det. the content of H_2O_2.

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(54) Title of the Invention: Test Paper for Determination of Hydrogen Peroxide Content

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Brief Explanation of the Graphs

Graph 1 shows the limit of determination of the testing paper by this invention.

Graph 2 shows the limit of determination of the testing paper which is removed the coloring matter.

Detailed Explanation of the Invention

This invention relates to the test paper for the determination of hydrogen peroxide content which is comprised by allowing hydrogen peroxide decomposing enzyme, o-tolidine, red coloring matter and pH buffer to be contained to a filter paper.

Hydrogen peroxide is widely used for bleaching, decoloring, sterilization or oxidation of fiber, pulp, food, chemical, etc., also for sterilization, decoloring or ablution of the various containers, apparatus, etc.

The usage purposes for food is to bleach raw noodle, dried noodle, fish-paste products such as boiled fish paste, fish cake, etc., wheat, barley, edible oil and fat, etc. However, when the residues of hydrogen peroxide in the aforesaid food products is high, it has been known that it causes poisoning. Especially, in foods which are eaten in a relatively short period after they are produced, the residue of hydrogen peroxide in the food products is sometimes fairly high, therefore it is necessary to give thorough attention. For that reason, the usage amount of hydrogen peroxide was specified by the Ministry of Welfare. According to this specification, for instance, less than 100 mg (100 ppm) per 1kg of raw wheat noodle (including packed noodle), boiled fish pastes, and less than 30 ppm per 1kg of herring roe and other food products have been specified. Therefore, the necessity to confirm the amount of residual hydrogen peroxide in food products has been increased. Conventional study of quantitative analysis and quantitative determination have been considerably done, for instance, there are color reaction method with dititanium sulfate, the potassium permanganate titration method, iodine titration method, color reaction method with vanadium sulfate, rhodan ammon method, method of using dissolved oxygen meter, method for detecting ultraviolet spectral sensitivity, etc.; in these, the first three are generally used. For instance, for the determination of hydrogen peroxide in raw wheat noodle by iodine titration method, raw wheat noodle is homogenized with a mixer for approximately 10 minutes, and its extracted liquid is separated with centrifugal separator, and a reagent such as potassium iodide or ammonium molybdate is added into said liquid

and it is allowed to stand for approximately 10 minutes, and then it is titrated with a sodium thiosulfate solution.

However, the aforesaid method is not suitable for the determination of hydrogen peroxide simply, quickly and accurately during the production procedure, at the time immediately before shipment, and at the time immediately before usage. Also, in the color reaction method with dititanium sulfate where the procedure is relatively simple, there is the disadvantage of being difficult to judge due to the light yellow color tone. Therefore a method which can obtain an accurate value in a short period, and a simple procedure has been strongly desired.

This invention is to present the test paper for the determination of hydrogen peroxide content which is able to determine an accurate value of the hydrogen peroxide content with simple procedures.

The method for the preparation of the test paper by this invention which is comprised by allowing hydrogen peroxide decomposing enzyme, for instance, peroxidase or catalase; o-tolidine; red coloring matter, for instance, acid fuchsine; and pH buffer, for instance, citrate buffer to be contained to a filter paper. However, this test paper is not limited to filter paper if the paper can contain the aforesaid reagents.

Next, a representative test paper by this invention will be explained.

The practically most suitable compounding ratio of the aforesaid reagents is:

hydrogen peroxide decomposing agent (peroxidase)	10 mg
o-tolidine (8% ethanol solution contains 1% hydrochloride)	12.5 mg
red coloring matter (acid fuchsine)	15 mg

pH buffer, for instance, 47.5 ml citrate buffer (0.9g citric acid, 5.4g sodium citrate).

A test paper is prepared by dipping a filter paper (for instance, Toyo filter paper No. 524) in the mixed solution of the aforesaid reagents for approximately 30 seconds, thereafter it is placed in a light screening bottle and dried at room temperature.

Next, regarding the usage method of the test paper prepared in this invention will be explained.

As the sample, a certain amount of wheat noodle is mashed in an earthenware mortar, and is made to an amount of 5 times the aforesaid amount of wheat noodle by adding water, and it is well stirred to become homogeneous.

The test paper by this invention is dipped into its suspension, thereafter the color tone of the test paper for 20 seconds after it is taken out is compared with a standard color tone chart which is previously prepared, and the amount of hydrogen peroxide content is determined.

The reaction mechanism for the coloring of the test paper in this invention will be explained according to the aforesaid practical example.

Hydrogen peroxide is decomposed by peroxidase; at this time, o-tolidine functions as the hydrogen donor, and the color of itself becomes blue. The darkness of the blue tone is increased proportional to the amount of hydrogen peroxide. Therefore determination of hydrogen peroxide is able to be done by usage of this mechanism.

However, the influence on the color tone by the enzyme strength of the peroxidase (or catalase) and the concentration of o-tolidine is indistinguishable visually, therefore a very small amount of hydrogen peroxide is unable to be detected. In this invention, the visibility of the aforesaid blue color is further increased, and with that a very small amount of hydrogen peroxide is able to be detected by usage of the aforesaid o-tolidine and peroxidase and with that, red color matter, for instance, acid fuchsine color matter.

Especially an amount of hydrogen peroxide of 1 ppm ~ 20 ppm (furthermore between 1 ppm ~ 10 ppm) is able to be distinguished by an extremely clear variation in color tone. The inaccuracy of determination between this is within ±2.5%.

An actual usage example of another test paper for the determination of hydrogen peroxide in this invention will be explained.

The treatment for the hydrogen peroxide of raw noodle is usually done by dipping said raw noodle into an approximately 300 ppm (or 400 ppm) solution tub for a minute. At this time hydrogen peroxide is added for replenishment, however if the concentration can be determined in a short period and accurately, it is very desirable for quality control.

When the amount of hydrogen peroxide in a 20 fold dilution of the aforesaid hydrogen peroxide solution in the tub is determined by the usage of the test paper in this invention, its value can be simply and accurately known.

Accordingly, in comparison to the conventional testing method, the effectiveness of this invention will be explained according to a test example where the test paper in this invention was used to the extracted liquid of wheat noodle and a test example where the limit of determination of the test paper in this invention was searched.

Test Example 1

By usage of the extracted liquid of wheat noodle, the determination value by usage of the iodine metric method and dissolved oxygen meter and the determination results by usage of the test paper in this invention were compared. The following six kinds of concentration of extracted liquid of wheat noodle were prepared.

No. 1 Handmade noodle was dipped into a 600 ppm hydrogen peroxide solution, and it was mashed, and then it was homogenized with 5 times water and its extracted liquid 1 was prepared.

No. 2 An extracted liquid 2 was prepared in a similar manner as the aforesaid No. 1, except a 1200 ppm hydrogen peroxide solution was used.

No. 3 Wheat noodle on the market was dipped into a 300 ppm hydrogen peroxide solution, and it was mashed, and then it was homogenized with 5 times of water, and its extracted liquid 3 was prepared.

No. 4 An extracted liquid 4 was prepared in a similar manner as extracted liquid 3, except a 600 ppm hydrogen peroxide solution was used.

No. 5 An extracted liquid 5 was prepared in a similar manner as extracted liquid 3, except a 900 ppm hydrogen peroxide solution was used.

No. 6 An extracted liquid 6 was prepared in a similar manner as extracted liquid 3, except a 1200 ppm hydrogen peroxide solution was used.

The amount of hydrogen peroxide in the aforesaid 6 kinds of extracted liquid were determined by usage of the iodine metric method and dissolved oxygen meter (manufactured by Denki Kagaku Keiki K.K.); its results are shown in Table 1.

Based on the determination value of hydrogen peroxide in Table 1, standard hydrogen peroxide solutions having concentrations of 17 ppm, 26 ppm and 30 ppm corresponded to extracted liquid 1,2 and 4 and the color tones which were colored by dipping the test papers by this invention into the aforesaid extracted liquid 1, 2 and 4 were compared, and the color tones colored with extracted liquid 1 and the 17 ppm standard hydrogen peroxide solution were matched. And also the color tones colored with extracted liquid 2 and the 26 ppm standard hydrogen peroxide solution, and the color tones of extracted liquid 4 and the 30 ppm standard hydrogen peroxide solution were matched.

As described above, the test paper by this invention can accurately determine the concentration of hydrogen peroxide when it is used to an extracted liquid of wheat noodle.

Table 1 Determination Results for the Concentration of Hydrogen Peroxide

Sample No.	Iodine Metric Method (ppm)	Dissolved Oxygen Meter (ppm)	Test paper by this invention
1	16.8	17	standard hydrogen peroxide solutions were prepared, and coloring with this solution and the extracted liquid of No. 1, No. 2, and No. 4 were confirmed, and it resulted in the color tones were matched
2	25.7	24	
3	14.3	16	
4	30.6	26	
5	63.4	Had to be diluted, otherwise impossible to determine	
6	64.6		

Test Example 2

Standard hydrogen peroxide solutions having concentration between 5 to 100 ppm which are already described were prepared, and the individual limit of determination was tested by usage of the test papers in this invention and the test papers which is removed the coloring matter.

The limit of determination in this invention means the range in which the coloring of the test paper in each concentration is able to be judged. The results are shown in Graph 1 and Graph 2.

In the graphs, the solid lines show the range which is able to be judged, and the dotted lines show the range which is unable to be judged. As shown in Graph 1, the test paper in this invention which is able to judge until 5 to 200 ppm by coloring corresponds to the concentration of hydrogen peroxide, but when the concentration of hydrogen peroxide becomes 30 ppm, its coloring becomes similar to the color tone of the concentration of 40 ppm, therefore it is impossible to judge; when the concentration of hydrogen peroxide becomes 50 ppm, it is able to be judged by the color tone. Also, the coloring at a concentration of 60 ppm and the color tone at a concentration of 90 ppm are similar, therefore it is unable to be judged, but coloring at a concentration of 100 ppm is able to be judged.

In the case of test paper which is removed the color matter, a concentration of 5 to 10 ppm of hydrogen peroxide is able to be judged by color tone, but at a concentration of 20 ppm and 30 ppm, judgment by color tone becomes impossible; of course judgment of more than this concentration is impossible. Like this, the test paper which is removed coloring matter becomes unable to be judged in the stage of a concentration 10 ppm lower than the test paper by this invention, and it shows the limit of determination is low.

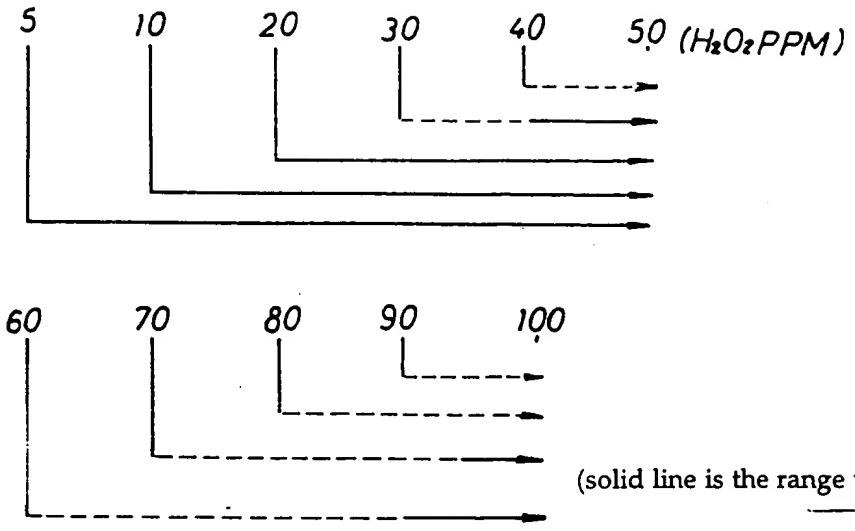
The test paper by this invention shows the most stable and accurate coloring when the sample has a pH in the range of 3.0 to 7.0.

The test paper for the determination of hydrogen peroxide content by this invention can be widely applied to the food product industries which use hydrogen peroxide and other various industries other than the application examples described above. As the former examples, it is effective for the determination of residual hydrogen peroxide after bleaching of dried egg, cheese, etc., also as the latter examples, it is effective for the determination of residual hydrogen peroxide after bleaching of fiber, pulp, wheat straw, bamboo skin, pig hair, button, glue, gelatin or starch. Also, it can be used for the determination of the residual amount of hydrogen peroxide after sterilizing container, etc., which is sterilized by usage of hydrogen peroxide.

(57) Claim

1. The test paper for the determination of hydrogen peroxide content which is comprised by allowing hydrogen peroxide decomposing enzyme, o-tolidine, red coloring matter and pH buffer to be contained to a filter paper.

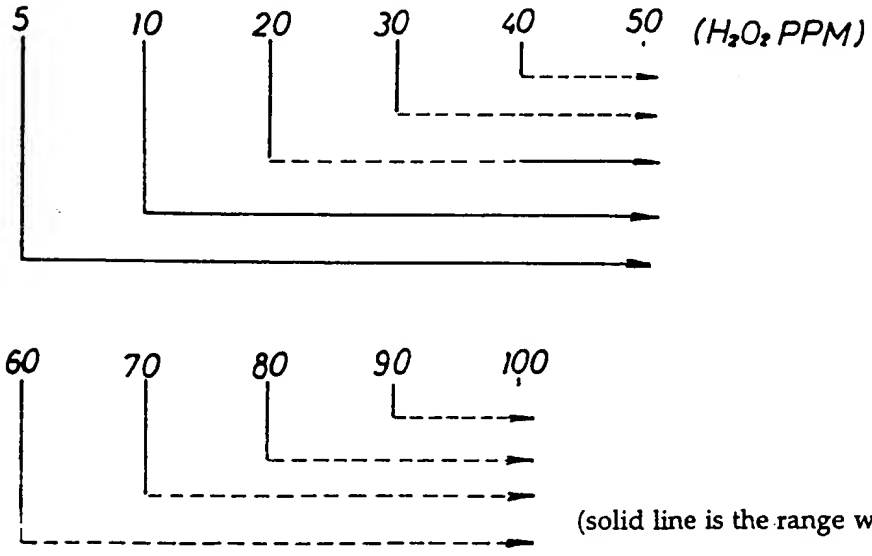
Graph 1



(solid line is the range which is able to judge)

(dotted line is the range which is unable to judge)

Graph 2



(solid line is the range which is able to judge)

(dotted line is the range which is unable to judge)

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⑭公告 昭和49年(1974)12月10日
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1

⑤④過酸化水素含量測定用試験紙

②特願 昭45-42726
②出願 昭45(1970)5月20日
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図面の簡単な説明

第1図は、本発明試験紙の定量限界を示すグラフである。

第2図は、色素を抜いた試験紙の定量限界を示すグラフである。

発明の詳細な説明

本発明は過酸化水素分解酵素、o-トリジン、赤色色素およびpH緩衝剤を濾紙に含有させてなる過酸化水素含量測定用試験紙に関するものである。

過酸化水素は繊維、パルプ、食品あるいは薬品などの漂白、脱色、殺菌あるいは酸化などに、また各種容器、器具などの殺菌、脱色あるいは洗滌などに広く用いられている。

食品への使用目的は主に生麺、乾麺、練製品類例えばかまぼこ、はんぺんあるいはちくわ等、小麦、押麦、食用油脂あるいは寒天などの漂白にある。しかるに、上記物質中に過酸化水素が高濃度残存するならば、これら食品を摂取した際に中毒を引き起こすということは、知られていることである。特に、製造後、比較的早い時期に摂取される食品においては過酸化水素の残存量もかなり多量に及ぶこともあり、充分な注意が必要となる。

2

それゆえに、過酸化水素の使用基準量が厚生省により制定されている。それによると、例えば生うどん(包装麺を含む。)、かまぼこおよびちくわなどにおいては製品1Kg当り100mg(100ppm)以下に、かずのこあるいはその他の食品においては30ppm以下の基準が定められている。従つて、食品中などに残存する過酸化水素量の確認の必要性が増して来た。

過酸化水素の定性および定量法は従来よりかなり研究され、例えば硫酸第二チタンによる呈色法、過マンガン酸カリ滴定法、ヨード滴定法、硫酸バナジウムによる呈色法、ロダンアンモン法、溶存酸素計を用いる方法、紫外分光度法あるいは検圧法などがあるが、それらのうち前三者の方法が一般に用いられている。ヨード滴定法により例えば生うどん中の過酸化水素を測定するには、生うどんをミキサーで約10分間ホモジナイズし、遠心分離機でその抽出液を分けとり、該液中にヨー化カリウムあるいはモリブデン酸アンモニウムなどの試薬を加え10分間放置し、その後、チオ硫酸ナトリウム溶液で滴定する。しかるに、製造工程中あるいは出荷直前または使用直前に簡単かつ迅速にそして正確に過酸化水素含量を測定するには上記方法は適当でない。また操作上比較的簡単な硫酸第二チタンによる呈色法においても、呈色の色調は淡黄色系統であるため判別しにくい欠点がある。故に、短時間で正確な値を得、操作も簡単である方法が強く望まれていた。

本発明は上記課題を解決せる簡易な操作で、かつ正確な値の測定可能な過酸化水素含量測定用試験紙を提供するものである。

本発明試験紙の調製方法は過酸化水素分解酵素例えばパーオキシダーゼあるいはカタラーゼおよびo-トリジンおよび赤色色素例えば酸性フクシンおよびpH緩衝剤例えればクエン酸バッファーなどを濾紙に含ませることよりなる。なお上記試薬含ませられるものであれば濾紙に限らない。

次に本発明試験紙の代表的な実施例を述べる。上記試薬の実用上最も適当である配分比率は

過酸化水素分解酵素
（パーオキシダーゼとして）　10㎎
o－トリジン（塩酸塩として1％を含む80％エタノール溶液）　12.5㎎
赤色色素　（酸性フクシンとして）　15㎎
PH緩衝剤例えばクエン酸バッファー　47.5㎖
（クエン酸0.9g、クエン酸ナトリウム5.4g）

である。

上記試薬の混合液中に濾紙（例えば東洋濾紙㈱524）を約30秒間浸し、その後、遮光ビンに入れ室温で乾燥して調製する。

本発明により調製された試験紙の使用方法に関して次に述べる。

一定量のうどんをサンプリングし、擂鉢ですりつぶし、水を加え上記うどん量の5倍量となし、均一になるように充分撹拌をする。その懸濁液に本発明による試験紙をつけ、引き上げ20秒後の色調を、予め作成しておいた標準色調表と比較し過酸化水素の含有量を測定する。

本発明の試験紙の発色反応機構を上記実施例に従つて次に述べる。

過酸化水素はパーオキシダーゼにより分解され、その際、o－トリジンは水素供与体として働き、それ自体は青色を呈するようになる。青色の色調は過酸化水素の量に比例して濃度を増す。故にこの機構を利用することにより過酸化水素量の測定が可能となる。

しかるに、パーオキシダーゼ（あるいはカタラーゼなど）の酵素力価および、o－トリジンの濃度による色調への影響は肉眼では区別がつきにくく、微量の過酸化水素を検出することは不可能である。ここにおいて本発明では、上記o－トリジンおよびパーオキシダーゼなどと共に赤色色素例えば酸性フクシン色素を用いることにより、上記青色の色調を更に鮮明にすると共に、微量の過酸化水素の検出も可能にしたのである。

特に従来不可能であつた1ppm～20ppm（更に1ppm～10ppmの間で）の過酸化水素量をも極めて鮮明な色調変化により判明可能にした。この間の測定誤差は±2.5％以内である。

本発明の過酸化水素測定用試験紙の別の実際の使用実施例を次に述べる。

生麺の過酸化水素処理としては通常300ppm（あるいは400ppm）程度の溶液槽に該生麺を寸時浸すことにより行なわれるのである。この際補充のため過酸化水素を加えるのであるが、短時間でかつ正確に濃度を測定出来るならば品質管理上大変望ましいのである。ここにおいて、上記過酸化水素槽液の20倍希釈液中の過酸化水素量を本発明に係る試験紙を用いて測定するならば、正確にしかも簡便に、その値を知ることが出来るのである。

なお、従来の試験法と対比して、うどん抽出液に本発明試験紙を用いた試験例および本発明試験紙の定量限界を調査した試験例によつて本発明の効果を説明する。

試験例　1

うどん抽出液を用いて、ヨードメトリー法および溶存酸素計による測定値と本発明試験紙による測定結果とを比較した。うどん抽出液は次の6種の濃度のものを調製した。

㈱1……600ppm の過酸化水素溶液に手製うどんを浸漬した後、うどんを5倍の水でホモナイズして抽出液1を調製した。

㈱2……1,200ppm の過酸化水素溶液を使用した以外はすべて前記㈱1と同様にして抽出液2を調製した。

㈱3……300ppm の過酸化水素溶液に市販うどんを浸漬した後、うどんを5倍の水でホモナイズして抽出液3を調製した。

㈱4……過酸化水素溶液を600ppm とした以外はすべて抽出液3と同様に行ない抽出液4を調製した。

㈱5……過酸化水素溶液を900ppm とした以外はすべて抽出液3と同様に行ない抽出液5を調製した。

㈱6……過酸化水素溶液を1200ppm とした以外はすべて抽出液3と同様に行ない抽出液6を調製した。

上記6種の抽出液の過酸化水素の量をヨードメトリー法および溶存酸素計（電気化学計器株式会社製）によつて測定し、その結果を第1表に示す。

第1表の過酸化水素の測定値にもとずき、抽出液1，2および4に対応する17ppm．26ppmおよび30ppm の濃度の標準過酸化水素溶液と前記抽出液1，2および4に本発明試験紙をつけて呈

色した色調を比較したところ、抽出液1と17 ppm の標準過化水素との呈色の色調は一致していた。同様に抽出液2と26 ppm 、および抽出液4と30 ppm の標準過酸化水素溶液との呈色※

※の色調も一致した。

以上のように、本発明の試験紙はうどんの抽出液に使用して正確に過酸化水素の濃度を測定することができる。

第 1 表　過酸化水素の濃度測定結果

試料 No	ヨードメトリー法 (ppm)	溶存酸素計 (ppm)	本発明試験紙
1	16.8	17	標準過酸化水素溶液を作成し、No 1, 2および4のうどん抽出液との呈色を比較したところ色調が一致した。
2	25.7	24	
3	14.3	16	
4	30.6	26	
5	63.4	希釈しなければ測定不可能	
6	64.6		

試験例　2

5～100 ppm の間の濃度既知の標準過酸化水素溶液を調製し、本発明試験紙と本発明より色素を抜いた試験紙を用いて夫々の定量限界を調査した。

本試験における定量限界とは、各濃度における試験紙の呈色によつて判別可能な区域をいう。

結果を第1図および第2図に示す。なお、図中の実線は判別可能区域を、点線は判別不可能区域をあらわす。

第1図によつて明らかなように本発明試験紙は、5より20 ppm までは過酸化水素の濃度に対応する呈色によつて判別することが可能であるが、30 ppm の濃度になるとその呈色は40 ppm とはその色調が類似して判別は不可能となり、50 ppm の濃度になるとその呈色の色調によつて判別可能になることを示している。

また、60 ppm の濃度の呈色は、90 ppm の呈色とは色調が似ており判別が不可能であるが、100 ppm の濃度の呈色になると判別が可能になることを示している。

これに対し、第2図の色素を抜いた試験紙の場合は、5～10 ppm までは過酸化水素の濃度を呈色の色調によつて判別が可能であるが、20 ppm の濃度では30 ppm とはその呈色の色調による判別は不可能となり、それ以上の濃度では勿論不可能である。このように色素を抜いた試験紙は、本発明の試験紙よりも10 ppm も濃度が低い段階で判別が不可能となり、その定量限界の低いことを示している。

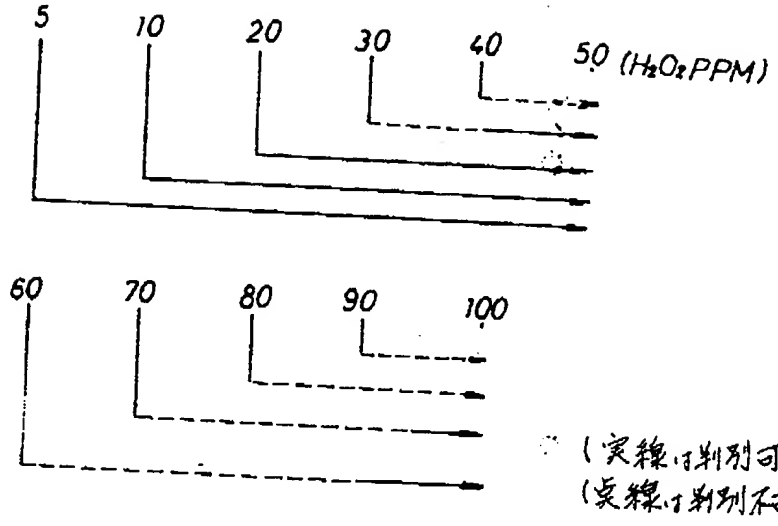
本発明の試験紙は検体のPH が3.0～7.0の範囲にあるときに最も安定でかつ正常の呈色を示す。

本発明の過酸化水素含量測定用試験紙は既述の応用例の外に過酸化水素を用いる食品工業およびその他の各種工業において、広く応用されうる。前者の例としては、乾燥卵やチーズなどの漂白作用後の残存過酸化水素の測定に、又、後者の例としては、繊維、パルプ、麦桿、竹皮、豚毛、ボタン、ニカワ、ゼラチンあるいはデンプン製造時の漂白作用後の残存過酸化水素の測定に有効である。その他、過酸化水素を用いての容器などの殺菌作用後の残存量測定にも使用出来る。

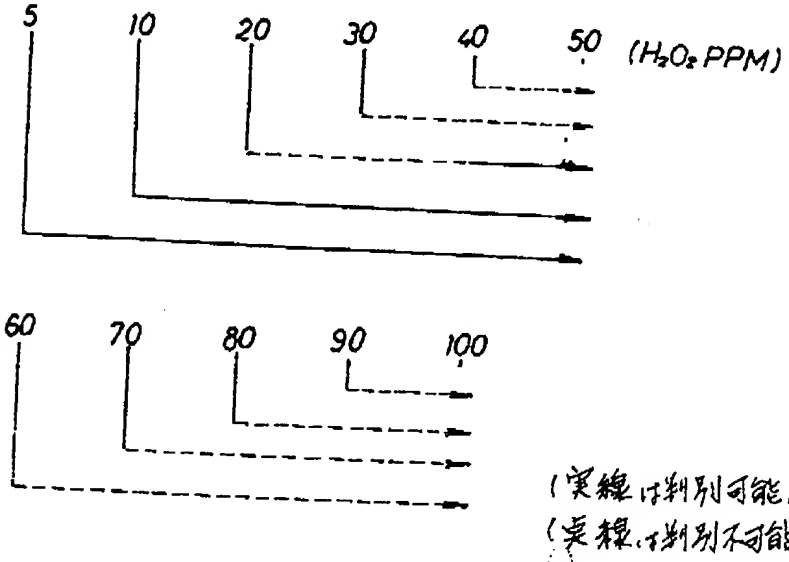
(57)特許請求の範囲

1　過酸化水素分解酵素、o-トリジン、赤色色素およびpH緩衝剤を濾紙に含有させてなる過酸化水素含量測定用試験紙。

第1図


5
10
20
30
40
50 (H_2O_2 PPM)
60
70
80
90
100
(実線は判別可能区域)
(点線は判別不可能区域)

第2図


5
10
20
30
40
50 (H_2O_2 PPM)
60
70
80
90
100
(実線は判別可能区域)
(点線は判別不可能区域)